

**CLAIMS:**

1. **A method of preparing a xenotransplantable porcine islet preparation capable upon xenotransplantation of producing porcine insulin in an appropriate recipient mammal**, the method including or comprising the steps of:
  - 5 (I) harvesting the pancreas of piglets at or near full term gestation, and
  - (ii) extracting pancreatic  $\beta$  islet cells from the harvested pancreaswherein the islets (at least at some stage in the performance of the method) are exposed to nicotinamide.
2. A method as claimed in claim 1 wherein the method includes or comprises the steps of:
  - 10 (I) harvesting the pancreas of piglets at or near full term gestation, and
  - (ii) preparing a culture of the pancreatic  $\beta$  islet cells
  - (iii) simultaneously with step (ii) and/or after step (ii) extracting pancreatic  $\beta$  islet cells from the culture of the harvested pancreasand the islets (at least at some stage in the performance of the method) are exposed to nicotinamide.
3. A method as claimed in Claim 1 or 2 where the piglets from which the pancreatic  $\beta$  islet cells are extracted are at from -20 to +10 days full term gestation.
4. A method as claimed in claim 3 wherein the piglets are at from -7 to +10 days full term. gestation.
5. A method as claimed in any one of the preceding claims wherein the extraction is performed using a suitable collagenase.
6. A method as claimed in claim 5 wherein the collagenase is selected from human Liberase® or porcine Liberase®.
7. A method as claimed in claim 6 wherein the collagenase is human Liberase®.
8. A method as claimed in any one of the preceding claims wherein the culture includes harvested pancreas in a supportive mammalian albumin substantially free of non-human microbiological agents.
9. A method as claimed in claim 8 wherein the mammalian albumin is human serum albumin (HSA).
10. A method as claimed in any one of the preceding claims wherein the islets are treated with nicotinamide after their extraction from the pancreas.

11. A method as claimed in any one of the preceding claims wherein the method includes the further step of treating the islets with IgF-1 or the N-terminal tripeptide of IgF-1 (GPE).
12. A method as claimed in claim 11 wherein the exposure to IgF<sub>1</sub> or to GPE is greater for those cells from piglets furthest from full term gestation.
13. A method as claimed in claim 11 or 12 wherein there is exposure to IgF<sub>1</sub> for all cells extracted irrespective of their relationship to full term gestation.
14. A method as claimed in any one of the preceding claims wherein the pancreas and/or islets are subject to a trauma protecting agent selected from suitable anaesthetic agents.
15. A method as claimed in claim 14 wherein the trauma protecting agent is lignocaine.
16. A method as claimed in any one of the preceding claims wherein step (iii) of the method includes mechanically reducing the harvested pancreas in the presence of the islet trauma protecting agent.
17. A method as claimed in any one of the preceding claims wherein an antibiotic is associated with the islet cells.
18. A method as claimed in claim 17 wherein said antibiotic is ciproxin.
19. **A method of preparing a xenotransplantable porcine islet preparation capable upon xenotransplantation of producing porcine insulin in an appropriate recipient mammal, said method including or comprising the steps of:**
- (I) harvesting the pancreas of piglets at or near full term gestation, and
- (ii) preparing a culture of the pancreatic  $\beta$  islet cells
- (iii) simultaneously with step (ii) and/or after step (ii) extracting pancreatic  $\beta$  islet cells from the culture of the harvested pancreas
- and
- (iv) encapsulating the islet cells with a biocompatible xenotransplantable material, said material **in vivo** being both glucose and insulin porous, wherein nicotinamide is introduced to the islets or islet cells prior to encapsulation at any one or more stages of the procedure.
20. A method as claimed in claim 19 wherein the piglets at or near full term gestation from which the pancreatic  $\beta$  islet cells are extracted are at from -20 to +10 days full term gestation.

21. A method as claimed in claim 20 wherein the piglets are at from -7 to +10 days full term gestation.
22. A method as claimed in any one of claims 19 to 21 wherein the extraction is performed using a suitable collagenase
- 5 23. A method as claimed in claim 22 wherein the collagenase is selected from human Liberase® or porcine Liberase®.
24. A method as claimed in claim 23 wherein the collagenase is human Liberase®.
25. A method as claimed in any one of claims 19 to 24 wherein the culture includes harvested pancreas in a supportive mammalian albumin substantially free of non-
- 10 human microbiological agents.
26. A method as claimed in claim 25 wherein the mammalian albumin is human serum albumin (HSA).
27. A method as claimed in any one of claims 19 to 26 wherein the islets are treated with nicotinamide after their extraction from the pancreas.
- 15 28. A method as claimed in any one of claims 19 to 27 wherein the method includes the further step of treating the islets with IgF-1 or the N-terminal tripeptide of IgF-1 (GPE).
29. A method as claimed in claim 28 wherein the exposure to IgF<sub>1</sub> or to GPE is greater for those cells from piglets furthest from full term gestation.
- 20 30. A method as claimed in claim 28 or 29 wherein there is exposure to IgF<sub>1</sub> for all cells extracted irrespective of their relationship to full term gestation.
31. A method as claimed in any one of claims 19 to 30 wherein the pancreas and/or islets are subject to a trauma protecting agent selected from suitable anaesthetic agents.
32. A method as claimed in claim 31 wherein the trauma protecting agent is lignocaine.
- 25 33. A method as claimed in any one of claims 19 to 32 wherein step (iii) of the method includes mechanically reducing the harvested pancreas in the presence of the islet trauma protecting agent.
34. A method as claimed in any one of claims 19 to 33 wherein an antibiotic is associated with the islet cells.
- 30 35. A method as claimed in claim 34 wherein the antibiotic is ciproxin.
36. A method as claimed in any one of claims 19 to 35 wherein the biocompatible material is a suitable alginate.

37. A method as claimed in claim 36 wherein the alginate is in ultra pure form.
38. A method as claimed in any one of claims 19 to 37 wherein each islet or grouping of islets is entrapped in an *in vivo* insulin and glucose porous biocompatible alginate or alginate-like surround.
- 5 39. A method as claimed in claim 38 wherein the encapsulation provides a surround which prevents, once implanted, direct tissue contact with the islets.
40. A method as claimed in claim 39 wherein each encapsulation involves presenting islets and a suitable alginate solution into a source of compatible cations thereby to entrap the islets in a cation - alginate gel.
- 10 41. A method as claimed in claim 40 wherein the cation alginate gel is calcium-alginate gel.
42. A method as claimed in claim 41 wherein the alginate used in the solution is sodium alginate, and the islet and sodium alginate solution is presented as a droplet into a bath of suitable cations.
- 15 43. A method as claimed in claim 42 wherein the islet and sodium alginate solution is of 1.6% w/w.
44. A method as claimed in claim 43 wherein the suitable cations are calcium chloride.
45. A method as claimed in claim 44 wherein the gel encased islets are coated with a positively charged material and thereafter are provided with an outer coat of a suitable alginate.
- 20 46. A method as claimed in claim 45 wherein the positive charging material is poly-L-ornithine.
47. A method as claimed in claim 46 wherein the gel entrapping the islets within the outer coating is then liquified.
- 25 48. A method as claimed in claim 47 wherein the liquification involves or comes about by the addition of sodium citrate.
49. A method as claimed in any one of claims 19 to 48 wherein the encapsulation produces capsules.
50. A method as claimed in claim 49 wherein the capsules contain a plurality of islet cells.
- 30 51. A method as claimed in claim 50 wherein the capsules contain substantially three islet cells.

52. A method as claimed in claim 50 wherein the capsules have a diameter of substantially from about 300 to 400 microns.
53. A method as claimed in claim 52 wherein following liquification of the alginate entrapping the islets there are the further steps of:
- 5 - washing the capsules
- further coating the capsules with alginate
54. A **xenotransplantable capsule** prepared according to the method as claimed in anyone of claims 1 to 53.
55. A **xenotransplantable preparation** being or including viable porcine islets prepared according to a method as claimed in anyone of claims 1 to 53.
- 10 56. A **xenotransplantable capsule** of at least one porcine pancreatic  $\beta$  islet cell comprising at least one viable porcine pancreatic  $\beta$  islet cell enclosed in an *in vivo* glucose porous and insulin porous biocompatible material.
57. A **method for treatment of a mammalian patient** suffering from diabetes which comprises:
- 15 (a) extracting pancreatic  $\beta$  islet cells from piglets at or near full term gestation;
- (b) Simultaneously with, and/or after (a), treating said islets with nicotinamide,
- (c) encapsulating said islets in a biocompatible material which will allow *in vivo* glucose movement to and insulin movement from the islets, and
- 20 (d) injecting or otherwise implanting the encapsulated islet cells of step (c) so as to transplant into said mammalian patient an effective amount of viable piglet islet cells capable of producing insulin in the patient,
58. A method as claimed in claim 57 wherein the method further includes the step of administering nicotinamide to the mammalian patient at least subsequent to
- 25 transplantation.
59. A method as claimed in claim 57 or 58 wherein the method further includes the step of prescribing to the patient, prior to or after the implantation step, a casein-free diet (as herein described).
60. A method as claimed in any one of claims 47 to 59 wherein the method further
- 30 includes the step of exposure of the pancreatic  $\beta$  islet cells at some stage after extraction from the piglets and prior to encapsulation to IgF<sub>1</sub> or to GPE.
61. A method as claimed in any one of claims 47 to 60 wherein the harvesting of the

- islets at least during any substantial confrontation (eg; mincing and/or enzymatic challenge) is in the presence of a trauma protecting agent.
62. A method as claimed in claim 61 wherein the trauma protecting agent is used during the isolation and/or preparation thereof for encapsulation.
- 5 63. A method as claimed in claim 62 wherein the agent is a trauma protecting agent is selected from suitable anaesthetic agents.
64. A method as claimed in claim 63 wherein the trauma protecting agent is lignocaine.
65. A method as claimed in any one of claims 47 to 62 wherein the patient prior to, during or after the step (d) has been subjected to a cholesterol lowering drug regime.
- 10 66. A method as claimed in claim 65 wherein the drug is of the "statin" family.
67. A method as claimed in claim 66 wherein the drug is pravastatin.
68. A method as claimed in any one of claims 47 to 68 wherein the yield of viable porcine islets obtained from the extraction of step a) is enhanced by the use of a suitable collagenase.
- 15 69. A method as claimed in claim 68 wherein the collagenase is selected from human Liberase® or porcine Liberase®.
70. A method as claimed in claim 69 wherein the collagenase is human Liberase®.
71. A method as claimed in any one of claims 47 to 70 wherein the extraction of step a) includes mechanical treatment of the islets.
- 20 72. A method as claimed in claim 71 wherein the mechanical treatment follows application of a suitable anaesthetic to the pancreatic tissue.
73. A method as claimed in claim 72 wherein the anaesthetic is lignocaine.
74. A method as claimed in any one of claims 47 to 73 wherein the piglets from which the pancreatic  $\beta$  islet cells are extracted are at from -20 to +10 days full term
- 25 gestation.
75. A method as claimed in claim 74 wherein the piglets are at from -7 to +10 days full term gestation.
76. A method as claimed in any one of claims 47 to 75 wherein the biocompatible material is a suitable alginate.
- 30 77. A method as claimed in claim 76 wherein the alginate is in ultra pure form.
78. A method as claimed in any one of claims 47 to 77 wherein each islet or grouping of islets is entrapped in an *in vivo* insulin and glucose porous biocompatible alginate or

alginate-like surround.

79. A method as claimed in claim 78 wherein the encapsulation provides a surround which prevents, once implanted, direct tissue contact with the islets.
80. A method as claimed in claim 79 wherein each encapsulation involves presenting islets and a suitable alginate solution into a source of compatible cations thereby to entrap the islets in a cation - alginate gel.
81. A method as claimed in claim 80 wherein the cation alginate gel is calcium-alginate gel.
82. A method as claimed in claim 81 wherein the alginate used in the solution is sodium alginate, and the islet and sodium alginate solution is presented as a droplet into a bath of suitable cations.
83. A method as claimed in claim 82 wherein the islet and sodium alginate solution is of 1.6% w/w.
84. A method as claimed in claim 83 wherein the suitable cations are calcium chloride.
85. A method as claimed in claim 84 wherein the gel encased islets are coated with a positively charged material and thereafter are provided with an outer coat of a suitable alginate.
86. A method as claimed in claim 85 wherein the positive charging material is poly-L-ornithine.
87. A method as claimed in claim 86 wherein the gel entrapping the islets within the outer coating is then liquified.
88. A method as claimed in claim 87 wherein the liquification involves or comes about by the addition of sodium citrate.
89. A method as claimed in any one of claims 47 to 88 wherein the encapsulation produces capsules.
90. A method as claimed in claim 89 wherein the capsules contain a plurality of islet cells.
91. A method as claimed in claim 90 wherein the capsules contain substantially three islet cells.
92. A method as claimed in claim 91 wherein the capsules have a diameter of substantially from about 300 to 400 microns.
93. A method as claimed in claim 92 wherein following liquification of the alginate

entrapping the islets there are the further steps of:

- washing the capsules

- further coating the capsules with alginate

94. **A method** for the treatment of a mammalian patient suffering from or predisposed to diabetes, said method including or comprising the steps of:
- (A) (i) harvesting the pancreas of piglets at or near full term gestation,
- (ii) culturing the harvested pancreas in Mammalian Albumin substantially free of non-human microbiological agents,
- (iii) simultaneously with step (ii) and/or after step (ii), extracting the islets from the harvested pancreas using a suitable Liberase®,
- wherein the islets (at least at some stage in the performance of (A)) are exposed to nicotinamide;
- (B) (i) encapsulating the islets prepared by (A) with a suitable encapsulation material that allows both glucose and insulin movement therethrough, and
- (ii) implanting the encapsulated porcine islets into the recipient mammal.
95. A method as claimed in claim 94 wherein the Liberase® is selected from human Liberase® or porcine Liberase®.
96. A method as claimed in claim 95 wherein the Liberase® is human Liberase®.
97. A method as claimed in any one of claim 94 to 96 wherein the extraction of step a) includes mechanical treatment of the islets.
98. A method as claimed in claim 97 wherein the mechanical treatment follows application of a suitable anaesthetic to the pancreatic tissue.
99. A method as claimed in claim 98 wherein the anaesthetic is lignocaine.
100. A method as claimed in any one of claims 94 to 99 wherein the method further includes the step of administering nicotinamide to the recipient mammal prior to or after the implantation step.
101. A method as claimed in any one of claims 94 to 100 wherein the method further includes the step of prescribing for the patient, prior to or after the implantation step, a casein-free diet (as described herein).
102. A method as claimed in any one of claims 94 to 101 wherein the method further includes the step of subjecting the patient prior to or after the implantation step to a



cholesterol lower drug regime.

103. A method as claimed in claim 102 wherein the cholesterol lowering drug is of the "statin" family

104. A method as claimed in claim 103 wherein the cholesterol lowering drug is pravastatin or simvastatin.

105. **Encapsulated pancreatic islets** of a kind useful in the method of any one of claims 57 to 104.

106. **A method of treating a mammalian patient predisposed to or suffering from diabetes** which involves the xenotransplantation into such patient a capsule as claimed in claim 56.

107. **A method of treating a mammalian patient predisposed to or suffering from diabetes** which involves the xenotransplantation into such patient a xenotransplantable preparation as claimed in claim 55.

108. **A method of preparing a xenotransplantable porcine islet preparation capable upon xenotransplantation of producing porcine insulin in an appropriate recipient mammal** substantially as herein described with reference to one or more of the Figures and/or Examples.

109. **A method for treatment of a mammalian patient** suffering from diabetes substantially as herein described with reference to one or more of the Figures and/or Examples.